Conclusions: 3D tissue culture of expanded human mastoid-derived periosteal progenitor cells in resorbable PGLA fleeces initiates bone and cartilage formation on the cellular and molecular level. The generation of different mesenchymal tissue makes 3D tissue cultured periosteal progenitor cells to promising candidates for the treatment of OA. Further investigations with periosteal progenitor cells from donors with OA were necessary for an autologous therapy strategy.

P387

EFFECT OF BLOOD ON THE MORPHOLOGICAL, BIOCHEMICAL, AND BIOMECHANICAL PROPERTIES OF NEO-CARTILAGE, SYNTHESIZED BY ISOLATED CHONDROCYTES PRE-SEEDED ONTO A BIOLOGICAL SCAFFOLD

C. Sosio[1], F. Boschetti[2], C. Bevilacqua[3], L. Mangiavini[1], C. Scotti[1], M. Buragas[4], G. Fraschini[1], A. Gigante[3], G.M. Peretti[1]
[1]Hospital San Raffele - Università Vita-Salute, Milan, Italy, [2]Laboratory of Biological Structure Mechanics, Politecnico of Milano, Milano, Italy, [3]University of Ancona, Ancona, Italy, [4]Yale University, Yale, CT

Purpose: The use of autologous chondrocytes seeded onto a biological scaffold represent a current valid tool for cartilage repair. However, the effect of the contact of the blood to the engineered construct is unknown. The aim of this work was to investigate the effect of blood contact on the morphological, biochemical and biomechanical properties of engineered cartilage.
Methods: Articular chondrocytes were enzymatically isolated from swine joints, expanded in monolayer culture and seeded onto collagen membranes for two weeks. Peripheral blood was obtained from animals of the same spices. The cell-seeded collagen membranes were placed in contact with the blood, diluted with medium, for three days ("blood" group). As controls ("control" group), some samples were left in medium, with no blood contact. Some samples were retrieved from cultures after the blood contact, some others were left in standard culture conditions for 3 more weeks. Samples were analysed grossly, histologically, biochemically (MTT analysis), and by biomechanical analysis under unconfined geometry.
Results: Upon retrieval, samples from both groups showed increasing dimensions and weights overtime, with higher mean values recorded for control group. Biochemical evaluation demonstrated a transient reduction of the mitochondrial activity due to blood contact. Histological evaluation demonstrated evident cartilage-like matrix production for both groups. Biomechanical data showed a reduction of the values in the early culture time, followed by a stabilization regardless the presence of the blood.
Conclusions: The results obtained from this study demonstrate that the contact to blood of the samples determine a transient reduction of the mitochondrial activity. However, the morphological parameters seem to show a continue production of the cartilage matrix components overtime. This tissue engineered cartilage structure is easily reproducible and it could represent a valuable model for studying the behaviour of different variables on the newly formed cartilage.

P388

NEW TISSUE GROWTH AFTER COLLAGEN MENISCUS IMPLANT (CMI) PLACEMENT INCREASES ACTIVITY LEVELS AFTER TWO YEARS

W.G. Rodkey, K.K. Briggs
Steadman Hawkins Research Foundation, Vail, CO

Purpose: Absence of meniscus tissue leads to decreased clinical function and activity levels. In a previous study, we reported significant correlation between actual measured amounts of meniscus tissue removed at meniscectomy and symptoms, function and activity 2 years after surgery. In particular, it was noteworthy that patients with >50% remaining meniscus tissue were significantly better in all categories measured than patients with <50% total meniscus tissue remaining. The purpose of the present study was to determine, prospectively, changes in Tegner activity levels from preoperative to 2 years postoperative in patients who received Collagen Meniscus Implants (CMI) and were documented to have >50% total meniscus tissue at 1-year relook arthroscopy.
Methods: In a randomized controlled investigational device clinical trial (Level of Evidence I), 138 patients 18 to 60 years old underwent partial medial meniscectomy and placement of a CMI to fill the meniscus defect. There were 64 acute (no prior meniscus surgery) and 74 chronic (1 to 3 prior partial meniscectomies on the involved meniscus) patients. At index surgery, meniscus defect size was measured with specially designed instruments, and the percent of meniscus loss was calculated based on these actual measurements. Relook arthroscopy was performed at 1 year on 124 patients (90% surgical follow-up), and percent total meniscus tissue (remnant + new tissue) was determined by making these same measurements and calculations. Patients were followed clinically for a minimum of 2 years after CMI placement. At each follow-up, all patients completed questionnaires, including a Tegner score to assess activity. We then determined changes in Tegner score from the index surgery to 2 years status post CMI in these patients.
Results: Of 124 relooks, 111 patients (90%) had >50% total meniscus tissue. In these patients, average Tegner activity scores improved by two levels from 3 to 5 from preoperative to 2 years status post CMI. This increased change in activity levels significantly correlated with total meniscus tissue >50% ($r=0.21$, $p=0.02$). These findings mirrored those we previously reported for partial meniscectomy patients in which >50% of the meniscus was maintained.
Conclusions: There is a significant correlation between change (increase) in Tegner activity levels over 2 years and percent total meniscus tissue in patients who receive the CMI as treatment for meniscus loss and have >50% total meniscus tissue. This study confirms the importance of preserving as much meniscus tissue as possible at the time of repair or meniscectomy. It clearly supports the potential positive benefits of regrowing or regenerating lost meniscus tissue to assist patients in regaining their activity.

P389

CHONDROGENIC PROGENITOR CELLS DERIVED FROM LATE STAGES OF HUMAN OSTEOARTHRITIS

S. Koelling, J. Kruegel, T. Lochte, N. Miosge
University Goettingen, Goettingen, Germany

Purpose: Osteoarthritis (OA) is such a widespread complication of old age that it is expected to become the fourth leading cause of disability by the year 2020. As a hallmark of the pathogenesis of late stages of the disease, in addition to the diseased chondrocytes (type 1 cells), type 2 cells emerge. While

investigating where these type 2 originate from, we discovered chondrogenic progenitor cells in OA tissue.
Methods: With the help of cell culture, in situ-hybridization, immunohistochemistry, Western blot, real time RT-PCR, micro array and FACS we have characterized the two cell types, as well as progenitor cells from human OA tissue in vivo and in vitro.
Results: In vivo, type 1 and type 2 cells show a fibroblast-like expression pattern with collagen type II and type I synthesis. Type 2 cells exhibit a faster re-differentiation in 3D alginate culture. This is indicated by an increased collagen type II expression already after one week, as well as increased amounts of sox-9. A subpopulation of type 2 cells also found in OA cartilage consists of progenitor cells positive for so-called stem cell markers, e.g. CD105, CD73 or STRO-1 with unregulated PTHrP receptor, upregulated RANTES and ADAM-TS5 and lower expression values for sox-9 than in type 2 cells. Depending on the substrate (collagen, fibronectin or Matrigel) on which they have been grown and depending on the gender of the patients, these progenitor cells exhibit a strong interindividual regulation, for example, of collagen type I, fibronectin and integrin alpha 5. In contrast, collagen type II, sox-9 and COMP remain unregulated. However, they can be differentiated into osteoblasts, adipocytes and chondrocytes in vitro.
Conclusions: Due to their OA origin, the type 1 cells remain diseased chondrocytes even without any pathological OA influences in 3D culture. The re-differentiation efforts of the type 2 cells lead to a more chondrogenic expression pattern in comparison to their in vivo status. This shows a more flexible adaptation to changing environmental conditions and, therefore, they behave more like progenitor cells.

P390

OPTIMIZING DIFFERENTIATION PROTOCOL OF HUMAN MESENCHYMAL STEM CELLS IN SUSPENSION CULTURE MODEL: DURATION OF CULTURE CORRELATES WITH EFFECTIVENESS

J.J. Kraft, G.R. Dodge
Bone and Cartilage Research Lab, AI duPont Hospital for Children, Wilmington, DE

Purpose: The prospect of isolating mesenchymal stem cells (MSC) and culturing these cells into a biomaterial for transplantation into the same patient provides viable option for cartilage repair. In this study we used our previously described suspension culture model using poly-HEMA coated plates. We tested the hypothesis that MSC can differentiate into chondrocytes in our suspension culture model and that early passage MSCs have a greater ability to undergo chondrogenesis.
Methods: "Low passage" cells (p3) were isolated and expanded on adherent flasks for six weeks; "high passage" cells (p9) were isolated and expanded on adherent 75cm^2 flasks for several months. These cultures were kept in control or chondrogenic medium (CM). In suspension culture cells self-aggregate. Cells in each culture type were maintained in DMEM with 10% FBS or CM (DMEM with 10 μl/mL ITS+premix, 50μg/mL ascorbic acid, 40μg/mL proline, 10ng/mLTGF-β, and 0.1μM dexamethasone). Real-time PCR was performed in triplicate using iQ SYBR Green, Genes: CD44, collagen types I (CI) and II (CII), aggrecan, perlecan, decorin, MMP3, MMP13, ADAMTS5, and GAP.
Results: Control biomasses averaged 0.9ug each while those in CM averaged 7.5ug each. DNA content for the chondrogenic group was double the control. This suggests that the eight-fold increase in weight is due more to substantial ECM production and hydration of ECM components than proliferation. After three weeks of CM culturing, in suspension culture C II was over 600 fold higher than the control. However, CII expression stayed the same in suspension cultures after one week of culturing in CM. Adherent cultures increased in response to $\sim$70 fold. Western blot confirmed the presence of CII in the three week cultured MSCs; the control showed no CII at the protein level. Interestingly, p9 cells showed a higher CII relative mRNA expression before the culturing began. These p9 MSCs showed no change in expression with different culture conditions.
CM decreased CI expression in p3 suspension culture MSCs but the opposite effect was observed in adherent cultures. CI was expressed in p9 cells the relative expression did not change significantly in comparison. CD44 was examined since its expression can indicate a normal chondrocyte phenotype. P3 adherent cultured MSCs in CM decreased expression of CD44. However, CM increased CD44 expression when cultured in suspension culture. With longer culture time in CM expression of MMP13 becomes much lower as CI and CII expression dramatically increases (in CM). In p3 cells, both aggrecan and ADAMTS-5 expression increases in CM suspension culture at one week however, when cultured for three weeks, ADAMTS-5 expression decreases and aggrecan expression remains 5-fold higher than the control. This supports an initial remodeling of ECM components but the overall biosynthesis is towards the formation of the ECM. Aggrecan is downregulated by CM on adherent plates, suggesting suspension culture is more conducive to chondrogenesis. P9 MSCs do not significantly alter aggrecan or ADAMTS-5 expression with culture or medium
Conclusions: P9 MSC show little ability to alter their phenotype in comparison to p3 MSCs. Suspension culture initially appears to inhibit differentiation however this conclusion is misleading and this culture condition is optimal for the promotion of chondrogenesis. It is likely that duration of culture is more important to see change because of the architectural differences between an adherent monolayer and a biomass. One week in culture seems to show the cells in a transitional stage, however longer time in culture show increased cartilage markers suggesting this may be a novel approach to engineering cartilage-like material.

P391

A NOVEL MODEL OF CHONDROCYTE PROGENITOR CELLS HARVESTED IN TISSUE CULTURES FOR THE HEALING OF LOCAL ARTICULAR LESION

G. Maor[1], G. Nierenberg[2]
[1]Anatomy and Cell Biology,Faculty of Medcine,Technion Institute of Technology, Haifa, Israel, [2]Unit for Arthroscopy and Sports Traumatology Rambam Health Care Campus, Haifa, Israel

Purpose: Damage to Hyaline cartilage in a large weight bearing joint caused by either trauma or primary focal degenerative changes (OA), if not treated, can lead to damage of the subchondral bone resulting frequently in pain, disability, and sever altered quality of life. Since cartilage tissue does not heal spontaneously, joint functional rehabilitation is based various temporary medical and surgical solutions. We have developed a novel chondrocytes cell culture originating from porcine cartilage tissue retrieved from the mandibular condyle of a newborn animal.
Methods: Porcine Chndrocytes, originating from a mandibular condyle of a new born animal, are a unique source of cartilage cells.Gradual collagenase seperation yields a homogenous chondrocyte population, which unlike other cartilge source-derived cells preserve the capability of spontanous differentiation into cartilage forming cells.
Results: Following a short period of intensive proliferation, these cells start to differentiate into polygonal shaped cells, expressing Cbfa1-the skeletal tissues specific transcription factor, type II collagen and cartilage proteoglycan; thus producing ingredients of genuine hyaline cartilage. The cultured chondrocytes also preserve their responsiveness toward local and systemic regulating